Smaller than predicted increase in aboveground net primary production and yield of field-grown soybean under fully open-air [CO₂] elevation

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Abstract

The Intergovernmental Panel on Climate Change projects that atmospheric [CO₂] will reach 550 ppm by 2050. Numerous assessments of plant response to elevated [CO₂] have been conducted in chambers and enclosures, with only a few studies reporting responses in fully open-air, field conditions. Reported yields for the world's two major grain crops, wheat and rice, are substantially lower in free-air CO₂ enrichment (FACE) than predicted from similar elevated [CO₂] experiments within chambers. This discrepancy has major implications for forecasting future global food supply. Globally, the leguminous-crop soybean (Glycine max (L.) Merr.) is planted on more land than any other dicotyledonous crop. Previous studies have shown that total dry mass production increased on average 37% in response to increasing [CO₂] to approximately 700 ppm, but harvestable yield will increase only 24%. Is this representative of soybean responses under open-air field conditions? The effects of elevation of [CO₂] to 550 ppm on total production, partitioning and yield of soybean over 3 years are reported. This is the first FACE study of soybean (www.soyface.uiuc.edu) and the first on crops in the Midwest of North America, one of the major food production regions of the globe. Although increases in both aboveground net primary production (17-18%) and yield (15%) were consistent across three growing seasons and two cultivars, the relative stimulation was less than projected from previous chamber experiments. As in previous studies, partitioning to seed dry mass decreased; however, net production during vegetative growth did not increase and crop maturation was delayed, not accelerated as previously reported. These results suggest that chamber studies may have over-estimated the stimulatory effect of rising [CO2], with important implications on global food supply forecasts.

Keywords: atmospheric change, dry mass, elevated [CO₂], FACE, global change, Glycine max, harvest index, plant growth, seed yield

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Introduction

The Intergovernmental Panel on Climate Change (IPCC) projects that atmospheric [CO₂] will increase by 50% over current levels by the middle of this century

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(Prentice *et al.*, 2001). Elevation of $[CO_2]$ invariably increases plant production, reproductive output and crop yield (Drake *et al.*, 1997; Curtis & Wang, 1998). From a summary of 436 prior studies, a doubling of $[CO_2]$ increased yields of C3 crops on average by 33% (Kimball, 1983). However, these studies were all conducted in protected environments: laboratory and field enclosures or open-top chambers, all of which can substantially modify the microclimate and could amplify the elevated $[CO_2]$ effects on production (McLeod & Long, 1999; Long *et al.*, 2004). Additionally,

many of these experiments were conducted in pots or with soil barriers that potentially alter responses to elevated [CO₂] (Arp, 1991). Free-air CO₂ enrichment (FACE) systems escape many of the limitations of chamber fumigation methods, providing elevation of [CO₂] in the field without otherwise altering the microclimate. In addition, they provide sufficient treatment area to meet agronomic trials criteria and allow sufficient area for the destructive harvests needed for growth analysis over the life of the crop (reviewed: Long et al., 2004). To date, only two large-scale and fully replicated FACE facilities have reported elevated [CO₂] effects on yields of C3 grain crops, wheat and rice, and both showed smaller increases than expected from earlier chamber studies. Over three growing seasons, rice grain yield was increased by 7-15% in elevated [CO₂] (Kim et al., 2003), and wheat grain yield increased by 8% in two growing seasons (Kimball et al., 1995). These FACE experiments increased [CO₂] by about 200 ppm above current ambient concentration, whereas the average increase was 350 ppm in the chamber studies surveyed by Kimball (1983). If a linear yield response to elevated [CO2] is assumed, then the expected yield increase in these FACE studies is about 19% based on the earlier chamber studies.

These discrepancies are important as results from chamber studies form the basis for projecting global and regional food supplies. On a global scale, the positive effect of rising [CO₂] on crop yields has been projected to offset yield losses resulting from other aspects of global change such as higher temperature and water availability. For example, an integrated Hadley Center assessment of climate-change impacts on agricultural productivity in the conterminous United States (US) predicted that forecast climatic change for 2090 would diminish yields in most of the northern US wheat belts in the absence of any direct effect of elevated [CO₂] (Izaurralde et al., 2003). When the direct effects of elevated [CO₂] were added, the combined result was a yield increase. However, this simulation assumed an increase of 350 ppm [CO₂] would increase yield by 33% (Izaurralde et al., 2003). If chamber experiments have overestimated the direct effect of increased [CO₂], then this will have a major impact on projections of future crop yields and have wider implications for extrapolations from chamber studies to agro-ecosystems in general. Modern wheat and rice cultivars are largely determinate, and thus the smaller than expected responses in FACE may be atypical of a broader range of crops and reflect limitation at the level of sinks for the additional carbon. Modern soybean cultivars grown in the Midwest US include many indeterminate cultivars and gain nitrogen via nodulation, creating and sustaining additional carbon sinks. Therefore, indeterminate soybean may provide a good test of whether lower than predicted responses to elevated $[CO_2]$ in FACE are a more general phenomenon.

In the conterminous US, the soybean-corn agroecosystem covered approximately 61.8 million hectares in 2003 (USDA, 2004) making it arguably the largest single ecosystem type. Globally, soybean is the most important dicotyledonous seed crop in terms of area planted and is a major source of protein for human consumption (FAO-UN, 2002). With the global human population currently over 6 billion, and projected to be more than 9 billion by 2050 (UN, 2001), quantifying effects of climate change on crops such as soybean are paramount to understanding future food security (Long, 1998; Parry *et al.*, 2004).

Numerous studies have shown that elevated [CO₂] increases photosynthetic efficiency (reviewed: Drake et al., 1997; Long et al., 2004), which increases the photoassimilate supply, thus fueling increases in dry mass and yield production. One current postulate of elevated [CO₂] studies is that nitrogen limits stimulation of dry mass accumulation, and thus species that are capable of fixing nitrogen are better able to benefit from increased [CO₂] (Rogers et al., 1998; Morgan et al., 2001). Newly applied statistical techniques, termed meta-analysis, have been used to synthesize numerous independent studies into a single response to elevated [CO₂] relative to current [CO₂]. In these summaries, leguminous species exhibited the greatest increase (56%) in dry mass when grown under 500-800 ppm [CO₂] (Jablonski et al., 2002), although Ainsworth et al. (2002) in conducting a meta-analysis of soybean found a mean 37% increase in total dry mass in response to growth at 689 ppm [CO₂], averaged across all the studies examined.

The soybean free air CO₂ enrichment (SoyFACE) facility (Rogers et al., 2004) provided a unique opportunity to test the hypothesis that net production and yield increase in response to elevated [CO₂] is less under open-air conditions. First, the soybean cultivar used is indeterminate and nodulating, thus minimizing the possibility of potential limitation by nitrogen and sinks for additional carbohydrate. Secondly, soybean is inbreeding (Gizlice et al., 1994; Cui et al., 2001; Li et al., 2001) and the site is relatively uniform in topography and soil. The homogenous nature of the crop in combination with a fully replicated (n = 4), randomized complete block experimental design, increased the likelihood of detecting subtle elevated [CO₂] treatment effects. Finally, the scale of the treatment plots provided sufficient space to conduct regular harvests to track dry matter production over the growing season and analyze the basis of response.

The overall objective of this study was to determine what increases in aboveground net primary production (ANPP) and yield occurred in soybean when grown in elevated [CO₂] under fully open-air field conditions across three entire growing seasons, and whether these increases were less than predicted from prior chamber studies. Our second objective was to establish, via sequential harvests at 2-week intervals, how production and partitioning were differentially affected with time and developmental stage across the growing season.

Materials and methods

Site description

The SoyFACE (www.soyface.uiuc.edu) facility consisted of a 32 ha field (South Farms, University of Illinois at Urbana-Champaign; 40°03′21.3″N, 88°12′3.4″W, 230 m elevation). The soil is a Drummer-Flanagan series (fine-silty, mixed, mesic Typic Endoaquoll), typically very deep and formed from loess and silt parent material deposited on the till and outwash plains. This site has been in continuous cultivation of arable crops for over 100 years. Soybean and corn are each planted in half of the field and are rotated annually, following typical contemporary Illinois agricultural practice. The previous year's crop residue was chopped with a mower followed by tillage with a rip chisel in the fall and conventional single-pass cultivator tillage in the spring. No fertilizer was applied prior to planting of the soybean as it is a nitrogen-fixing crop, although some residual nitrogen was likely carried over from the application to the corn of the previous year (see Leakey et al., 2004). For an extended description of site including micrometeorology and climate, see Leakey et al. (2004) and Rogers et al. (2004).

SoyFACE elevated [CO₂] treatments consisted of eight octagonal plots within the 16 ha planted with soybean (for extended site and operation description see Ainsworth et al., 2004). The rings were separated by 100 m to avoid cross-contamination. Within each block, one control plot was at the current ambient [CO2] of 370 ppm and one plot was fumigated from sunrise to sunset to a target [CO₂] of 550 ppm, using the FACE design of Miglietta et al. (2001). Fumigation began 3 days after planting and operated over the remainder of the growing season until the crop was ready for harvest. The actual [CO₂] averaged across all three growing seasons was 551 ppm. The elevated [CO₂] treatment was precise as demonstrated by the 1 min averages of [CO₂], which varied less than $\pm 20\%$ from the target concentration for 96% of the time (T. Mies, personal communication).

Planting

Together with the surrounding field, the plots were planted using a mechanical seed planter to an approximate field density of 200 000 plants ha⁻¹. Indeterminate soybean (Glycine max (L.) Merr.) cultivars of maturity group type 3 were planted on day of year (DOY) 143 in 2001, DOY 152 in 2002 and DOY 147 in 2003 (May 23, June 1 and May 27, respectively). The cultivars used were Pana (2001, Illinois Foundation Seeds, Champaign, IL, USA) and Pioneer 93B15 (2002 & 2003; Pioneer Hi-Bred, Des Moines, IA, USA). Row spacing was 0.38 m (15 in.). In 2002 and 2003, plots were oversown by hand on the day of planting and thinned after crop emergence to approximately 20 plants m⁻¹ row to ensure uniform density. In 2001, the crop was treated on DOY 191 with a postemergent herbicide to control weeds (Boundary, Syngenta Crop Protection Canada Inc., Guelph, ON, Canada). A general insecticide (Sevin®, Bayer CropScience, Research Triangle Park, NC, USA) was used to control insect infestations in 2001 and 2002. In 2002 and 2003, the crop was treated with a preemergent herbicide (Boundary) and again postemergence in 2003 on DOY 175. On DOY 198, 2003, a hail storm defoliated 60% of the soybean crop (O. Dermody, personal communication) and destroyed 21% of the shoot dry mass; however, the shoots regrew to produce a final yield close to the annual average for the region. Slightly more dry mass was lost in the elevated [CO₂] treatment, but this was not statistically significant (current $[CO_2]$ 45.1 g m⁻² vs. elevated $[CO_2]$ 48.6 g m⁻²).

Harvesting

Biweekly over the growing season, subplots were randomly chosen with the constraint that sampled plots could not be adjacent to subplots harvested previously. Single buffer rows were left between harvested rows and two buffer rows or 30 cm of row length were left between harvested areas and the 30 cm wide walkways that allowed access to the crop. In 2001, two subplots ($30 \text{ cm} \times 38.1 \text{ cm}$, centered on a row) were taken at each harvest. In 2002, one larger subplot (60 cm × 38.1 cm, centered on a row) was harvested on each date to increase the uniformity in the number of plants per subplot and prevent damage to the crop during the subsequent destructive soil sampling (see Rodríguez, 2004). In 2003, subplot size was increased to $100 \,\mathrm{cm} \times 38.1 \,\mathrm{cm}$. Stems were cut within $0.5 \,\mathrm{cm}$ of the soil surface and all plants were sealed into a cooled storage container to prevent wilting and to minimize respiratory loss of mass. Counting, sorting and transfer to the drying oven of all subsamples was completed within 6 h of cutting.

Developmental stage was reported according to the system described by Ritchie et al. (1997). For each plant within each subplot, the number of leaves, branches, pods and nodes was determined. The following criteria were applied to determine whether an organ should be included in a count. Leaves were counted if they were open trifoliates, following Ritchie et al. (1997), in which at least one leaflet remained and in which $\geq 50\%$ of the area was green; if less than 50% was green it was included in litter. Unopened trifoliates were included in stem dry mass, but their mass contribution was negligible (<1%). Pods were included in the determination of number and the dry mass assessment if they contained at least one seed that was more that 3 mm in length (Ritchie et al., 1997); otherwise they were left with stem matter. Branches were counted provided they had at least one node with an attached leaf or a petiole scar. Node number was counted and plant height measured from the cut base to the topmost node bearing an open trifoliate. Normally, the primary shoot was the tallest, but following destruction of most apical meristems on the primary stems in the 2003 hailstorm, node number was counted and plant height was measured to the topmost node bearing an open trifoliate on the highest branch (apical replacement). Plants from each subplot were subsequently separated into stems and petioles, leaf tissue (based on the above criteria), and pods and then these separated components were placed into drying bags. Litter was captured in 60 cm × 38 cm galvanized steel mesh baskets placed between two rows in 2002 and 2003. The baskets were supported 3 cm above the soil to minimize decay and litter was collected biweekly. The litter and live plant components were dried in a forced-air oven at 65 °C for at least 4 days and until constant dry mass. For 2002 and 2003, ANPP was calculated as the sum of the dried leaf, stem, pod dry matter and cumulative litter dry mass. Immediately following the 2003 hailstorm, litter was collected from a 4 m² subplot within each plot, washed in large tubs to remove soil and dried to determine loss. Most (>95%) consisted of leaf and stem freshly stripped from the plants by the hail.

Random subsamples of 100 pods from each subplot were taken to assess the number of seeds per pod. After drying, subplot samples were mechanically threshed to remove pod husks from seeds. The seeds were redried for 1–2 days and seed mass was determined. From the threshed seeds, a subsample of 200 seeds from each subplot was weighed to calculate the individual seed mass. At crop maturity, final yield was assessed by harvesting a large (12.8 m²) subplot within each plot, from which no plant material had been removed during the growing season. All shoot dry mass was harvested by hand and threshed mechanically to separate seed

and all other shoot components, the ratio of seed to total (seed and shoot components) given as the harvest index (HI).

Statistical analysis

Effects of elevated [CO2] on seed yield, HI and cumulative litterfall were assessed with a randomized complete block mixed model analysis of variance (PROC MIXED, SAS v8.01, SAS Institute, Cary, NC, USA) with treatment as the fixed effect ($\alpha = 0.1$). The impact of not finding a difference between current and elevated [CO₂] would have greater repercussions than falsely finding an effect; therefore, we chose to minimize type II errors by using a 10% probability of committing a type I error. For all other parameter comparisons, a repeated measures mixed model analysis of variance (PROC MIXED) was used with DOY, treatment and the DOY by treatment interaction as fixed effects ($\alpha = 0.1$). Years were analyzed separately because of difference in cultivars (2001 vs. 2002 and 2003), crop rotation altering field position (2002 vs. 2001 and 2003), and defoliation caused by hail (2003). For all dry mass and plant growth parameters that were recorded throughout the growing season (e.g. stem mass and node number), all harvest dates within a year were included in the analysis. For dry mass and plant parameters that developed in later stages (e.g. seed mass and pod number), the analysis was conducted from the date of first occurrence through the end of the growing season. The best-fit variance/covariance matrices were chosen for each variable using Akaike's information criterion (Keselman et al., 1998; Littell et al., 1998, 2000). For both analyses, treatment effects are reported as the least-squared means and the associated variances are the difference in least-squared means between treatments calculated from a priori pairwise comparisons ($\alpha = 0.05$).

Results

Crop area

Although a different cultivar was used in 2001 and a hailstorm decreased final seed yield per unit ground area by about 40% in 2003 from the 2002 yield, the relative increase in seed yield because of elevated [CO₂] was remarkably similar across the 3 years: 16%, 15% and 15% in 2001, 2002 and 2003, respectively (Fig. 1 and Table 1). Equally consistent was a significant decline in harvest index (the proportion of shoot dry mass allocated to seed) of 3%, 2% and 2%, respectively (Fig. 1). These results were based on the final harvests of 12.8 m² plot. When harvests from smaller subplots

were used to follow the progression of the components of shoot dry mass over the growing season, significant increases in shoot, leaf, stem and pod dry mass because

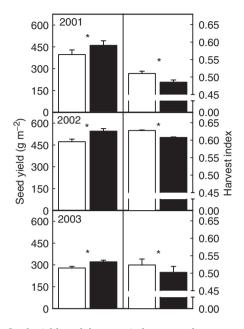


Fig. 1 Seed yield and harvest index over three seasons for *Glycine max* (cv. Pana-2001; cv. Pioneer 93B13-2002 and 2003) grown under current or elevated [CO₂]. Stars denote significant main effects differences ($\alpha \le 0.1$). Each bar, \pm the standard error of the difference between treatments, is based on four replicate blocks. These data are from harvests of 12.8 m² subplots within each replicate plot at maturation of the crop.

of elevated [CO₂] were evident only in the later stages of the growing season, with the largest differences in stem mass (Fig. 2). In 2002, elevated [CO₂] stimulated cumulative litterfall significantly (P = 0.03, $258 \,\mathrm{g}\,\mathrm{m}^{-2}$ vs. $355 \,\mathrm{g}\,\mathrm{m}^{-2}$), but not significantly in 2003 (P = 0.36, $287 \,\mathrm{g}\,\mathrm{m}^{-2}$ vs. $333 \,\mathrm{g}\,\mathrm{m}^{-2}$). ANPP was significantly increased by 15% and 17% in 2002 and 2003, respectively (Table 1), but again these differences were only evident in the later part of the growing season (Fig. 3).

The proportion of shoot biomass in seed at final harvest, HI, was significantly lower in elevated [CO₂] in all 3 years (Table 1). During pod-filling (growth stages R5 and R6, Ritchie et al., 1997), aboveground dry mass partitioning to reproductive production was significantly decreased in all three growing seasons in elevated [CO₂] (Fig. 4, Table 1). In 2001, the reduction in proportion of aboveground dry mass in pods resulted from retention of a larger leaf dry mass. In 2002 and 2003, the decreased partitioning of dry mass to pods appeared to result from a greater retention of mass (nonsignificant) in both leaves and stems. During the vegetative growth stage in 2001 and 2003, partitioning of dry mass between stems and leaves was not detectably affected by [CO₂]; however, in 2002 there was a significant increase in the proportion allocated to leaves vs. stems (data not shown). During flowering (R2 to R3, \sim DOY 215–220), partitioning between stems and leaves was not significantly different between plants grown in current and elevated $[CO_2]$.

Table 1 Field-grown Glycine max responses to elevated [CO₂] (550 ppm)

	2001			2002			2003		
	Treat	DOY	$Treat \times DOY$	Treat	DOY	$Treat \times DOY$	Treat	DOY	$Treat \times DOY$
Large subplot									
Seed yield (g m ²)	0.0915	NA	NA	0.0067	NA	NA	0.0312	NA	NA
Harvest index (dimensionless)	0.0305	NA	NA	0.0009	NA	NA	0.0934	NA	NA
Small subplot									
ANPP $(g m^{-2})$	_	_	_	0.0037	< 0.0001	0.0004	0.2465	< 0.0001	0.4344
Shoot dry mass (g m ⁻²)	< 0.0001	0.0168	0.0015	0.0091	< 0.0001	0.0047	0.1982	< 0.0001	0.4219
Stem dry mass (g m ⁻²)	< 0.0001	0.0592	0.0005	0.0198	< 0.0001	0.0640	0.1167	< 0.0001	0.1555
Leaf dry mass (g m ⁻²)	0.0002	0.0274	< 0.0001	0.0047	< 0.0001	0.5948	0.1606	< 0.0001	0.3903
Litterfall (g m ⁻²)	_	_	_	0.0071	< 0.0001	0.2147	0.3179	< 0.0001	0.7434
Mass per seed (g)	0.1832	< 0.0001	0.0040	0.5011	< 0.0001	0.3883	0.1780	< 0.0001	0.0496
Stem proportion	0.4506	< 0.0001	0.5214	0.0249	< 0.0001	0.0005	0.1651	< 0.0001	0.0010
Leaf proportion	0.7311	< 0.0001	0.0210	0.5079	< 0.0001	0.1676	0.2291	< 0.0001	0.0289
Pod proportion	0.0458	< 0.0001	0.4695	0.0199	< 0.0001	0.3314	0.0252	< 0.0001	0.0793

P-values from analysis of variance of fixed effects on measured growth and dry mass in current and elevated [CO₂] over the growing season. Parameters reported on a per unit ground area basis. Aboveground net primary production (ANPP) is the sum of shoot dry mass per square meter and cumulative litterfall per square meter. The small subplot P-values were calculated from a repeated measure, mixed model analysis of a randomized complete block experimental design. Bold values indicate significance at $\alpha \le 0.1$. Dashes indicate missing data and NA indicates not applicable since these samples were taken on a single day of year (DOY).

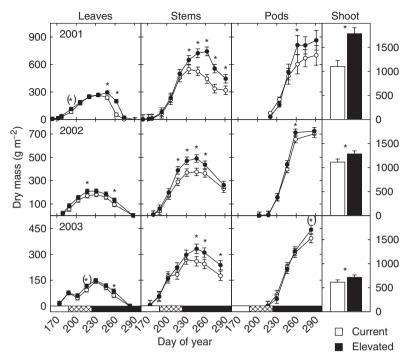


Fig. 2 Average aboveground dry mass by plant organ over three seasons for *Glycine max* (cv. Pana-2001; cv. Pioneer 93B15-2002 and 2003) grown under current or elevated [CO₂]. Leaf, stem and pod dry mass per square meter from small plot harvest over the growing season and shoot dry mass on day of year 262, 2001, 258, 2002 and 259, 2003 are reported. The average stage of development for each date is represented by the horizontal bar as follows: vegetative (white), flowering (R1, cross-hatched) and pod filling (R4, black). Each point is based on four replicate blocks and the error bars (some are obscured by symbol) are \pm the standard error of the difference between treatments within each date. Significant ($\alpha = 0.05$) and marginally significant ($\alpha = 0.1$) pairwise linear contrasts between treatments within each date are denoted by stars and bracketed stars, respectively.

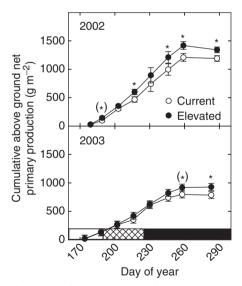


Fig. 3 Aboveground net primary production (ANPP) over two seasons for *Glycine max* cv. Pioneer 93B15 grown under current or elevated $[CO_2]$. The horizontal bar is as reported for Fig. 2.

Individual plants

The numbers of reproductive organs per plant were significantly stimulated by elevated [CO₂] in 2001 and

2002 (Table 2). In 2001, elevated $[CO_2]$ increased seed yield (Fig. 1) by increase in the number of seeds per pod (Table 2). However, the increase in 2002 and 2003 resulted from an increase in both the number of pods per plant and seeds per pod. Mass per seed was not increased by elevated $[CO_2]$ in any of the three years.

Elevated [CO₂] altered stem development late in the growing season in all years. In parallel with dry mass, greater height only became evident in the latter half of the growing season and coincided with the formation of additional nodes at this stage (R7 to R8) in elevated [CO₂] (Fig. 5). Continued growth of nodes in elevated [CO₂] likely explains the increased stem dry mass and height, while the additional leaves associated with these nodes may explain a statistically significant extension of the growing season by 2-7 days across the three years (F. Dohleman, unpublished data). Variations in the response of branch number per plant to elevated [CO₂] were likely because of differences in cultivars and loss of the primary apical meristem in 2003 (Table 2). The cultivar Pana (2001) had a lower number of branches (<1 plant⁻¹), which did not differ with [CO₂] treatment, whereas the cultivar 93B15 in 2002 had significantly more branches (control 1.1 ± 0.10 plant⁻¹ vs. elevated [CO₂]

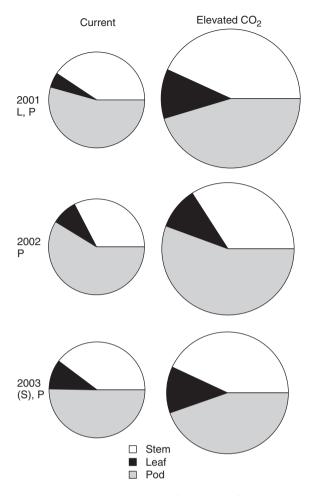


Fig. 4 Proportional distribution of mass to leaf, stem and pod dry mass on the harvest date with the greatest shoot dry mass (day of year 262, 2001, 258, 2002 and 259, 2003). Shoot dry mass as reported in Fig. 2 and the sizes of the pie graphs of the elevated [CO₂] treatment are normalized to the current atmospheric treatment within each year. Proportional distribution to organ types tested for differences between treatments within years was calculated from pairwise linear contrasts, and letters under each year indicate significant (α = 0.05) differences and letters within parentheses indicate marginally significant effects (α = 0.1; L, leaf; S, stem; P, pod).

 $1.6 \pm 0.10 \ \mathrm{plant}^{-1}$), but not in 2003 when destruction by hail resulted in large subsequent branching in both treatments (Table 2).

Discussion

When grown under fully open–air conditions in a FACE fumigation system, stimulation of ANPP and yield by elevated [CO₂] were significant, but smaller than predicted from open-top chamber studies (reviewed by Ainsworth *et al.*, 2002). Elevated [CO₂] increased the ANPP of field grown soybeans by 15–16% (Fig. 3). The large-scale final harvest showed an increase in seed

yield of about 15% in each growing season (Fig. 1). However, HI was decreased significantly by about 3% in elevated [CO₂], which is consistent with a decreased harvest index resulting from increased shoot dry mass as reported previously for a range of soybean cultivars (Amthor et al., 1994; Heagle et al., 1998; Ziska & Bunce, 2000). From the statistical summary of published reports of the elevated [CO₂] response of soybean within chambers, shoot dry mass was approximately 30% greater in plants grown in 450–550 ppm [CO₂] than in current [CO₂] (Ainsworth et al., 2002). It seems unlikely that our lower than expected stimulation is a function of the genotype used, given the limited genetic variation in soybean (Gizlice et al., 1994; Li et al., 2001). Further, similar responses were observed in all three years, even though a different cultivar was used in the first year. More importantly, the smaller than expected increase in yield of rice and wheat in FACE, relative to earlier chamber studies, was repeated with soybean. Although the response found here for soybean is half that predicted from the meta-analysis of soybean, the overall yield stimulation by elevated [CO₂] is greater than in rice (7-15%, Kim et al., 2003) and spring wheat (8%, Kimball et al., 1995). This difference between FACE experiments may reflect the indeterminate nature of the soybean cultivars grown and/or the nitrogen fixing capacity of sovbean (Ainsworth et al., 2004).

Holtum & Winter (2003) recently suggested that the high-frequency fluctuations in [CO₂] of the type produced by FACE technology may diminish the response of photosynthesis to elevated [CO₂]. However, this seems an unlikely explanation of the lower than expected stimulation observed in the FACE crop experiments. First, Hendrey et al. (1997) found no difference between constant and fluctuating elevated [CO₂] on photosynthesis in wheat leaves, providing that oscillations had a half-cycle of <30 s, which would include most of the fluctuations observed in FACE systems. Secondly, large fluctuations in [CO₂] are also observed in open-top chambers (McLeod & Long, 1999), which are much of the database on effects of elevated [CO₂] on yield (e.g. Ainsworth et al., 2002). Our findings confirm those of prior FACE studies with other grain crops, that coefficients accounting for the direct effects of elevated [CO₂] based on chamber studies are likely leading to an overestimation of the effects of global change on food supply.

There was a notable lack of response early in the lifecycle of field-grown soybean, although stimulation of final dry mass and yield occured in this study and previous open-top chamber studies. Previous studies have shown that elevated [CO₂] effects are apparent in soybean during early vegetative growth and sustained through the duration of the experiment (Ziska & Bunce,

0.0038

0.0002

0.8405

0.0501

< 0.0001

< 0.0001

< 0.0001

< 0.0001

0.2230

0.5979

0.8742

0.8681

0.4654

< 0.0001

< 0.0001

< 0.0001

< 0.0001

0.7658

2001 2002 2003 Treat DOY $Treat \times DOY$ Treat DOY $Treat \times DOY$ Treat DOY $Treat \times DOY$ Individual plants < 0.0001 0.4002 Height (cm) 0.0623 0.0632 0.1339 < 0.0001 0.2305 < 0.0001 0.3134

0.0001

0.0049

0.1809

0.0390

Table 2 Individual plant-level responses to elevated [CO₂] (550 ppm)

0.0005

< 0.0001

< 0.0001

< 0.0001

< 0.0001

0.9471

0.0098

0.3769

0.0021

0.0304

0.9235

0.0169

0.7558

0.4686

0.0263

Branches (number)

Nodes (number)

Leaves (number)

Seed/pod (number)

Pods (number)

P-values from analysis of variance of fixed effects on measured growth of field-grown *Glycine max* in current and elevated [CO₂] over the growing season. Parameters are expressed on a per plant basis. The P-values were calculated from a repeated measure, mixed model analysis of a randomized complete block experimental design. Bold values indicate significance at $\alpha \le 0.1$. Dashes indicate missing data.

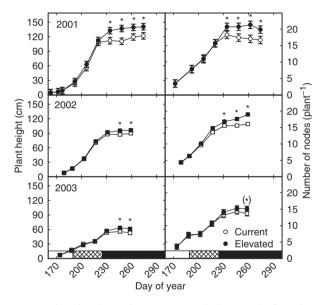


Fig. 5 Plant height to the topmost node bearing leaflets that have unrolled/unfolded (edges not touching) or bearing a flowering raceme, over three growing seasons *Glycine max* (cv. Pana-2001; cv. Pioneer 93b13-2002 and 2003). The number of nodes was determined as the total node number including cotyledon node through the topmost node with unrolled/unfolded leaflets. Horizontal bar is as reported for Fig. 2.

1995; Miller et al., 1998). The greatest stimulation of dry mass due to elevated [CO₂] was found to occur during flowering, declining through pod filling (Ainsworth et al., 2002). Contrary to these findings, no significant increase in any growth parameter was observed until pod filling in SoyFACE in any of the three years. Furthermore, the subsequent relative stimulation by elevated [CO₂] was constant throughout pod filling to maturity. These findings suggest that studies of plant responses to elevated [CO₂], that are limited to the vegetative stage, may be misleading as to the relative

effect of elevated [CO₂] on annual ANPP and seed yield.

0.2444

0.4021

0.9870

0.6207

0.8716

In indeterminate soybean, additional carbon resources may be used to sustain growth in stem length and leaf production, which occurs in these cultivars after flowering has begun (Ainsworth et al., 2004). Under elevated [CO₂], node formation continued later into the season (2-7 days) adding one to three additional nodes per plant (Fig. 5). This continued growth resulted in taller plants, potentially leading to increased crop lodging thereby decreasing harvestable yield. This delay contradicts previous studies in chambers that have reported no effect (Ziska et al., 1998) or accelerated maturity (Prior & Rogers, 1995; Miller et al., 1998) when soybean is grown in elevated [CO₂]. Had crops in open-top chambers shown this delayed senescence instead of accelerated maturity, the apparent yield increase difference between chambers and FACE may have been even greater. In the fieldgrown soybean in the present study, elevated [CO₂] significantly delayed maturation in both cultivars and in all three years. This may explain the increased leaf dry mass late in the growing season, which was particularly evident in 2001 (Fig. 2). Longer leaf retention in elevated [CO2] likely results in greater net carbon accumulation for field grown plants, which would sustain dry mass production throughout pod filling in elevated [CO₂]. Unfortunately, this extension of the growing season increases the risk of an early frost killing of pods before completion of seed filling.

Under field fumigation with elevated [CO_2], the seed yield increase (15%) was because of increased pods per plant and increased seeds per pod (Tables 1 and 2), not the mass of individual seeds. This is also consistent with results of previous chamber studies (Heagle *et al.*, 1998; Ainsworth *et al.*, 2002). During pod filling in all three years, partitioning of dry mass to pod mass, and

therefore harvest index, was significantly decreased by elevated [CO₂] with concomitant increases in stem partitioning (Fig. 4 and Table 2). Similar decreases in harvest index have been found in previous studies of soybean grown under elevated [CO₂] in chambers (Heagle *et al.*, 1998; Ziska *et al.*, 1998; Ainsworth *et al.*, 2002). This possibly results from sustained vegetative growth in elevated [CO₂] and suggests that potential for further increases in harvestable yield exists if stem carbon allocation can be repressed and re-apportioned to seeds.

Conclusion

In common with other FACE studies of grain crops, soybean show a substantially smaller increase in yield when grown in elevated [CO₂] under fully open-air conditions than observed in previous chamber studies. A prolongation of the growing season caused by elevated [CO₂] in open-air conditions, is also counter to the previously reported lack of effect or shortened time to crop maturation. Elevated [CO₂] did not cause the significant early season stimulation in vegetative growth and dry mass observed in chamber studies, which brings into question the use of response data of annuals to elevated [CO2] in vegetative growth as a proxy for mature plant response. However, other qualitative responses of soybean to elevated [CO₂] are similar in both this fully open-air treatment and chamber treatments. Both techniques show an increase in net primary production, but a decrease in harvest index without altering individual seed mass. Most importantly the results suggest that our current projections of future supply of food grains based on the increase in yield observed in chamber studies may be overly optimistic. Resolving this potential overestimation of global food supply will require further studies of this type with major food crops, and side-by-side comparisons of the different technologies for examining the effects of elevated [CO₂] on crops.

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